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A Peristaltic Pump Driven ^{89}Zr Separation Module

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Abstract. To facilitate the separation of ^{89}Zr produced in yttrium foils, an automated separation module was designed and assembled. The module separates more than 85% of produced ^{89}Zr – activity in 3 g foils in less than 90 min. About 10 % remains in the dissolving vial. The quality of the separated ^{89}Zr activity was investigated for labeling of the HER2-binding monoclonal antibody fragment, trastuzumab-Fab.

Keywords: ^{89}Zr, PET, Separation Module, Automation.
PACS: 28.60.+s, 87.57.uk, 87.57.un

INTRODUCTION

^{89}Zr (β^+=22.7 %, $t_{1/2}$=3.27 d) is a useful radiometal for immuno-PET, the *in vivo* imaging and quantification of monoclonal antibodies (mAbs) with positron emission tomography (PET). This is because ^{89}Zr has relatively high β^+ intensity and a physical half-life suitably matched to the pharmacokinetics of mAbs *in vivo* (days). Since the first utilization of ^{89}Zr for labeling of intact antibodies was presented,[1] ^{89}Zr has been given a great deal of attention.[2]

^{89}Zr is accessible with hospital cyclotrons through the ^{89}Y(p,n)^{89}Zr reaction on natural yttrium (^{89}Y =100 %). One common procedure to separate ^{89}Zr from irradiated yttrium material is to use a solid-phase hydroxamate resin[3,4] from which ^{89}Zr is eluted with oxalic acid as [^{89}Zr]Zr-oxalate. In 2010 Vosjan et al.[5] published a protocol describing the step by step procedure, where separated [^{89}Zr]Zr-oxalate is directly used to label mAbs or other proteins pre-conjugated with the commercially available bifunctional chelate p-isothiocyanatobenzyl-desferrioxamine (Df-Bz-NCS).

This work focuses on the separation of ^{89}Zr activity from natural yttrium target material with a hydroxamate resin. For this purpose, an automated module was designed and assembled. An automated separation module is one important parameter in the streamlining of regular ^{89}Zr-production. Additionally, personnel doses are decreased with an automated module.

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MATERIAL AND METHODS

Equipment & Materials

The module consists of a peristaltic pump (Welco, 6-12 VDC) and 6 dual pinch valves (Takasago, 12 VDC) syringes and hydroxamate resin, which are mounted on an aluminum plate (Figure 1). On the upper side of the pinch valves, the syringes and the hydroxamate resin are connected with Luer Lock tips to a Pharmed BPT tube (1 mm i.d and 3 mm o.d). On the lower side of the pinch valves the tubes are connected to a common line with 3 way stopcocks (only used as tee-unions).

Pinch valves and pump are connected via an on/off 8 channel USB relay card (Quancom-USBREL8/LC) to 6-12 VDC lines. A PC lap-top with a LabVIEW (National Instruments) program is used to control the relay card. The separation program starts a sequence which consists of several steps. Each step has a user specified valve/pump setting and duration (Figure 2).

To decrease metal contamination low metal level HCl (Merck 30 % Ultrapur®) and water (Sigma Aldrich, Traceselect®) is used. MeCN is pretreated with chelex 100 resin (Bio-Rad Laboratories) to remove metal ions.

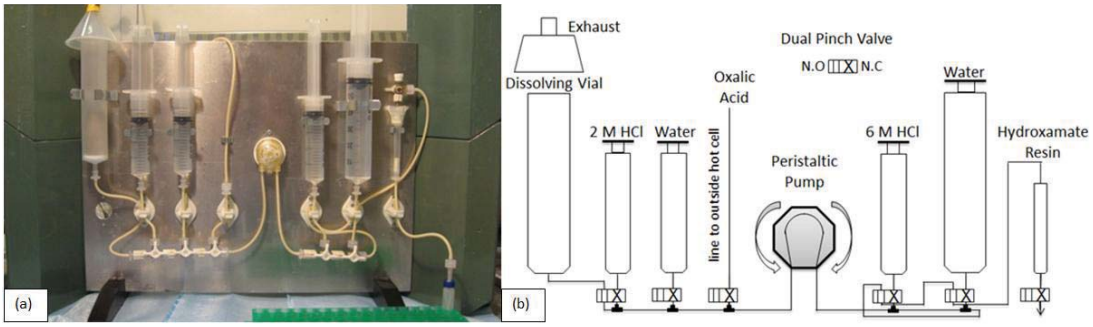

FIGURE 1. (a). The module loaded with resin, liquids and an irradiated foil in the dissolving vial. (b). Schematic overview of the set up.

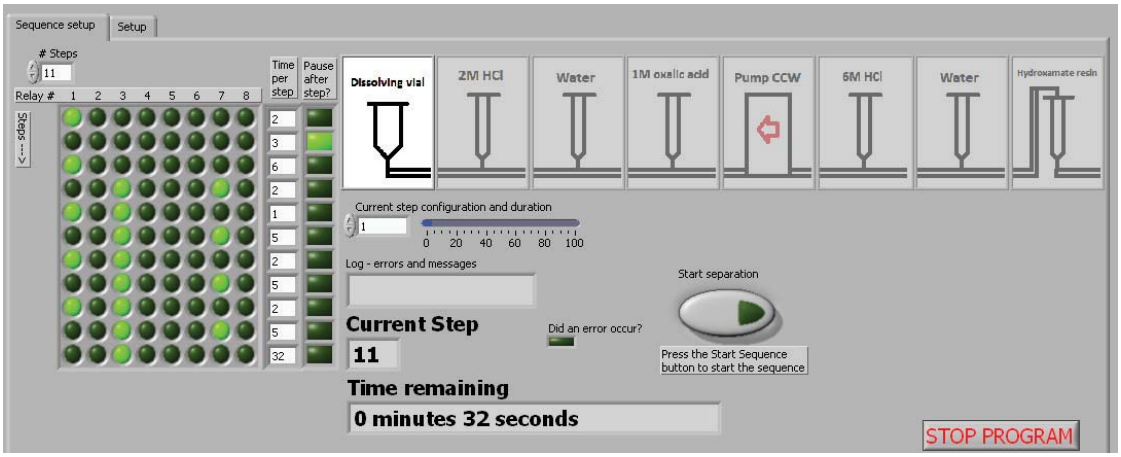

FIGURE 2. LabVIEW Valve/Pump control-program

Irradiation

^{89}Zr is normally produced in ~50x20x0.64 mm, ~3g, yttrium foils (Alfa Aesar 99.9%) with 45 µA, ~12.8 MeV protons with a home-made solid multi-purpose target system.[6] One hour irradiation results in 2150 ± 197 MBq (n =13).

Separation

The automated separation in this work is performed based on the separation described by Holland et al.[7] Briefly, a home manufactured 100 mg hydroxamate resin is manually activated, with MeCN, water, 2 M HCl and connected to the module. Also water and HCl-syringes are connected to the module before an irradiated yttrium foil is placed in the dissolving vial. The peristaltic pump is placed so that liquids on the right side of the pump can go to vials on the left side of the pump and vice versa by reversing the direction of the pump rotation i.e. switch the polarity of the motor. The program is started where 6 M HCl is added to dissolve the foil. Because of approximately ten times larger target masses in this work compared to Holland et al.,[7] water and HCl volumes are increased proportionally. Due to the violent reaction with HCl, foils are dissolved in several steps by pumping 1 ml HCl to the dissolving vial and waiting 75 sec before next addition (total of ~18 ml). To collect fumes the exhaust is operating during the entire dissolving step by circulating water through a water jet vacuum pump. In the next step, water is added to ensure a <2 M HCl concentration. The solution is transferred to the resin where ^{89}Zr is trapped. The resin is then washed with 2 M HCl and water to remove Y(III) ions and other impurities. The final step is to elute ^{89}Zr with 1 M oxalic acid.

An elution distribution profile was determined with 10 x 200 µl 1 M oxalic acid injections into the module with a pump speed of ~ 3.5 ml/min (Figure 3). To see any difference in separation trends for a smaller foil, the same procedure was also performed for irradiated 1.5 g foils. Elution with oxalic acid can be performed using either with a prefilled syringe connected to the module or via an external line out from the hot cell to the lab as in Figure 1. The latter gives the operator more flexibility if several [^{89}Zr]Zr-oxalate batches are to be delivered.

Labeling

The separated activity from the third 200 µl fraction elute was used to label trastuzumab-Fab and tested against HER2 expressing SKOV-3 cells according to Siikanen et al.[8] The trastuzumab-Fab is obtained by fragmentation using a FragIT kit (Genovis, Lund).

Specific activity

To calculate specific activity, the amount of cold zirconium was measured with inductively coupled plasma optical emission spectrometry (ICP-EOS) with a detection limit of 0.5 ppb for zirconium. For the ICP-EOS analysis the first 1 ml eluted oxalic acid separated from one un-irradiated foil was analysed.

RESULTS

Separation of ten 200 µl [^{89}Zr]Zr-oxalate fractions took less than 90 min. More than 85% of the activity measured in foils was collected in total of 2 ml oxalic acid. No remarkable differences in elution profiles were observed for a 1.5g and a 3g foil separation. Approximately 90% of the activity transferred to the resin was eluted in the first milliliter of oxalic acid (Figure 3).

The overall labeling yield of ^{89}Zr-Df-trastuzumab-Fab was over 99%. The radioconjugate was stable in PBS for at least 2 weeks and retained its binding specificity to HER2 receptors *in vitro* (Figure 4).

The ICP-EOS indicated a mass of 1.1 µg of cold Zr.

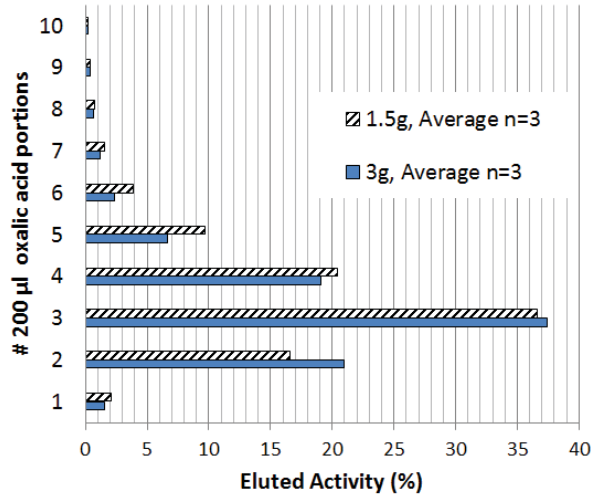

FIGURE 3. Elution profile averaged over three separations

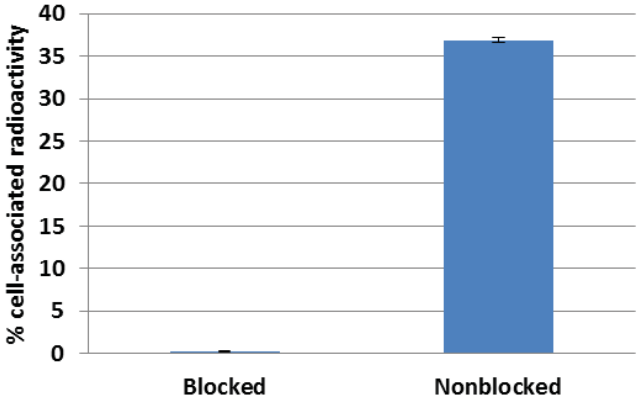

FIGURE 4. Binding specificity of ^{89}Zr-trastuzumab-Fab on HER2-expressing SKOV-3 cells. ^{89}Zr-trastuzumab-Fab binds specifically to HER2 receptors in SKOV-3 cells as shown by a pre-saturation of excess of cold trastuzumab (blocked) before addition of labeled substance

DISCUSSION AND CONCLUSIONS

In this work a relatively large foil has been used, about 5-10 times more yttrium than common at other radiopharmaceutical production sites. Approximately 10 % of foil activity was trapped in the dissolving vial. This loss is expected to be lower for sites with smaller target masses which mean smaller dissolving volumes.

One hour irradiation yields about 2.1 GBq ^{89}Zr in a 3g foil. Approximately 1.8 GBq (85%) of foil activity is eluted in 2 ml oxalic acid. About 1.6 GBq (90%) of total eluted activity is eluted in the first 1 ml oxalic acid fraction according to several separations like the example in Figure 3. Provided that irradiation conditions do not increase the amount of cold zirconium the specific activity of ^{89}Zr is expected to be about 1.5 GBq/µg of zirconium (1 h irradiation). However more extensive studies involving specific activity and metal contamination measurements have been initiated to examine the separated product more thoroughly.

We have designed a simple and inexpensive automated separation module which also reduces personnel doses. This is helpful for sites which aim to distribute large amounts of ^{89}Zr activity. The system is flexible and can be useful for other similar tasks if the setup and relay-pump sequences are reconfigured. Relay card, pump and pinch valves cost about 100 € each. All syringes, 3-way stopcocks and tubes in contact with liquids and foil are disposables.

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